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600 E. MERMAID LANE
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Title: Interventions to Ensure the Microbial Safety of Sprouts

Author(s): W. F. Fett,

Citation: In" Microbiology of Fruits and Vegetables" ed. by G.M. Sapers, J. R. Gorny, and A. E. Yousef CRC, Taylor and Francis Group, Boca Raton, FL (2006) Chapter 8 Page 187-209

Number: 7563

8 Interventions to Ensure the Microbial Safety of Sprouts

William F. Fett

CONTENTS

8.1 INTRODUCTION

Sprouts are considered a natural healthy food by many consumers in the U.S. and elsewhere. The North American sprouting industry has grown rapidly from only a very few commercial growers in 1970 to approximately 300 growers today with a total product market value of approximately $250,000,000 [1]. Over 20 seed types are used for sprouting in commercial operations and in the home [2]. Commercial sprouting operations are indoor facilities and in the U.S. are usually small in size with less than 10 employees [3]. Distribution of sprouts to retail outlets is local or regional.

Sprouts can be classified as either green sprouts or bean sprouts. Green sprouts such as alfalfa, clover, broccoli, radish, and sunflower have been subjected to light at some point in the growing process to allow for chlorophyll development. Bean (mung bean and soybean) sprouts are propagated under continuous dark and thus do not produce chlorophyll. Mung bean sprouts make up the major portion of the market for sprouts in the U.S. Green sprouts

Mention of trade names or commercial products in this chapter is solely for the purpose of providing specific information and does not imply recommendation or endorsement by the U.S. Department of Agriculture.

are consumed raw while bean sprouts are most often, but not always, served after at least light cooking.

Unfortunately, since 1995, both in the U.S. and in other countries, there have been numerous outbreaks of foodborne illness due to the consumption of sprouts contaminated with the bacterial pathogens salmonella and *Escherichia coli* O157 [4,5]. Raw sprouts were identified as a special food safety problem due to the potential for bacterial human pathogens to multiply from low levels on contaminated seed to high levels on sprouts due to favorable conditions of moisture, temperature, and nutrient availability during the sprouting process [4]. The U.S. Food and Drug Administration (FDA) has released a number of consumer advisories informing the consuming public about the risks associated with eating raw sprouts, the latest occurring in November 2003 [6], and raw sprouts are considered a "potentially hazardous food" in the FDA Food Code [7]. The consumer advisory states: "Those persons who wish to reduce the risk of foodborne illness from sprouts are advised not to eat raw sprouts." Particularly vulnerable to foodborne illness are the young, the elderly, and the immunocompromised.

This chapter provides an overview of the incidence and causes of sprout-related foodborne illness, interventions that have been tested for eliminating human pathogens from seeds and sprouts, means for reducing the risk of future outbreaks, and finally, further research needs.

8.2 FOODBORNE ILLNESS ASSOCIATED WITH SPROUTS

Several foodborne human pathogens have been isolated from sprouts and consumption of contaminated sprouts has been associated with numerous outbreaks of foodborne illness in the U.S. (Table 8.1). Some of these outbreaks have been international in scope due to the international distribution of sprout seed [10,12,24,25]. In addition to those in the U.S., sprout-related outbreaks of foodborne illness have been reported in several other countries including Canada, Japan, Sweden, Denmark, Holland, Finland, and the U.K. [4,5]. The earliest documented outbreak in the U.S. occurred in 1973 and was associated with consumption of raw sprouts grown with home sprouting kits containing soybean, cress, and mustard seed contaminated with enterotoxigenic *Bacillus cereus* [8]. There were no additional sprout-related outbreaks of foodborne illness recorded in the U.S. until 1990. Since 1995 there have been many outbreaks due to contamination of alfalfa and clover sprouts with various serovars of salmonella or *E. coli* O157. The first foodborne outbreak due to mung bean sprouts in the U.S. occurred in 2000 due to contamination with salmonella [9]. Previously, the only documented mung bean-associated outbreak of salmonellosis took place in England and Sweden in 1988 [26]. The number of culture confirmed cases in the U.S. has ranged from less than 10 to over 400 per outbreak. The actual number of cases was most likely much higher due to the significant underreporting normally encountered for

TABLE 8.1
Incidence of Foodborne Illness Due to Contaminated Sprouts in the U.S.

Year	Bacterium	Location	Sprout type	No. of culture confirmed cases	Ref.
1973	*Bacillus cereus*	TX	Soybean, cress, mustard	4	8
1990	*Salmonella* Anatum	WA	Alfalfa	15	9
1995	*Salmonella* Stanley	17 states/Finland	Alfalfa	242	10
1995	*Salmonella* Newport	OR	Alfalfa	69	9
1995–1996	*Salmonella* Newport	7 states/Canada/ Denmark	Alfalfa	>133	5, 11, 12
1996	*Salmonella* Stanley	VA	Alfalfa	30	9
1996	*Salmonella* Montevideo/ Meleagridis	CA/NV	Alfalfa/clover	492	13
1997	*Salmonella* Infantis/Anatum	KS/MO	Alfalfa	109	5
1997	*Escherichia coli* O157:H7	Multistate	Alfalfa	85	9, 14
1997–1998	*Salmonella* Senftenberg	CA/NV	Alfalfa/clover	60	13
1998	*Salmonella* Havana/Cubana	Multistate	Alfalfa	40	13, 15
1998	*Escherichia coli* O157:NM	CA/NV	Clover/alfalfa	8	13
1999	*Salmonella* Mbandaka	Multistate	Alfalfa	87	9, 16
1999	*Salmonella* spp.	MI	Alfalfa	34	9
1999	*Salmonella* Typhimurium	CO, CT	Alfalfa/clover	119	9, 17
1999	*Salmonella* Saint Paul	CA	Clover	36	9
1999	*Salmonella* Muenchen	Multistate	Alfalfa	~157	18
2000	*Salmonella* Enteritidis PT33	Multistate	Mung bean	75	9, 19
2001	*Salmonella* Kottbus	Multistate	Alfalfa	31	20
2001	*Salmonella* Enteritidis PT1	HI	Mung bean	26	21
2001	*Salmonella* Enteritidis PT913	FL	Mung bean	35	9
2002	*Escherichia coli* O157:H7	CA/NV	Alfalfa	5	18
2002	*Salmonella* Enteritidis	ME	Mung bean	16	22
2003	*Salmonella* Saint Paul	OR/WA	Alfalfa	8	22
2003	*Escherichia coli* O157:NM	CO/WY	Alfalfa	13	22
2003	*Escherichia coli* O157:H7	MN	Alfalfa	5	23
2003	*Salmonella* Chester	OR	Alfalfa	24	22
Salmonella	Bovismorbificans	Multistate	Alfalfa	28	22
Escherichia coli	O157:NM	GA	Alfalfa	5	22

foodborne illnesses [27]. The first recognized sprout-related outbreak due to *E. coli* O157:H7 occurred in Japan in 1996 and was associated with contaminated Daikon radish sprouts. To date this is the largest recorded foodborne outbreak due to contaminated sprouts worldwide with well over 7000 confirmed cases [28,29]. The first recorded sprout-related outbreak of foodborne illness in the U.S. due to contamination with *E. coli* O157:H7 was in 1997 [14]. Contaminated sprout seed is thought to be the primary source of the pathogens responsible for most sprout-related outbreaks of foodborne illness [4,5]. This conclusion is based on direct isolation of pathogens from seed of implicated lots and/or epidemiological evidence.

Several studies have indicated that salmonella and *E. coli* O157:H7 present initially on artificially as well as naturally contaminated seed have the potential to increase up to 10,000-fold on sprouts propagated at 20 to 30°C. The majority of growth of salmonella and *E. coli* O157:H7 on sprouting seed occurs during the first 48 hours. For sprouts grown from artificially inoculated seed, maximum populations of salmonella and *E. coli* O157:H7 ranging from 5 to 8 $\log_{10}$ colony-forming units (CFU)/g have been reported [30–39]. The maximum pathogen population obtained was not dependent on the initial inoculum level present on the seed [36]. For comparison, populations of total aerobes reported for sprouts typically range from 7 to 9 $\log_{10}$ CFU/g [30,40–42]. For salmonella on alfalfa, the doubling time was estimated at 47 minutes during the initial rapid growth phase and growth was not dependent on pathogen serovar, isolation source, or virulence [33]. Populations of salmonella and *E. coli* O157:H7 were stable from 48 hours to harvest at 3 to 5 days and then declined only slightly during subsequent storage of contaminated alfalfa sprouts at 5 to 9°C for 6 to 10 days [33,34,37]. Populations of *B. cereus* on sprouts grown from naturally contaminated alfalfa and mung bean seed reached approximately 4 $\log_{10}$ CFU/g [43]. The maximum pathogen populations attained during germination and growth of naturally contaminated seed under commercial practice may be several $\log_{10}$ units less than that for artificially inoculated seed [44]. Maximum populations of salmonella attained on alfalfa sprouts grown from two different lots of naturally contaminated seed were only 2 to 4 $\log_{10}$ MPN/g for salmonella. The reduced growth may be due to several factors. The first is the much lower overall contamination levels on naturally contaminated seed when compared to even the lowest initial pathogen populations utilized for laboratory studies. Second, pathogen populations on naturally contaminated seed may contain a higher percentage of injured cells. Third, differing methods of irrigation and increased irrigation frequency employed in commercial operations may affect the final pathogen populations attained. Interestingly, salmonella serovars attach more tightly to surfaces of alfalfa sprouts than do strains of *E. coli* O157:H7 and the difference in strength of attachment was proposed to explain, at least in part, the greater number of outbreaks of foodborne illness associated with contaminated sprouts due to salmonella [39].

Studies in several independent laboratories have indicated that bacterial human pathogens can be internalized in sprouts. By use of immunofluorescence and scanning immunoelectron microscopy, *E. coli* O157:H7 was located in stomata and the vascular system of radish sprouts grown from inoculated seed [45]. Bioluminescent *Salmonella* Montevideo and various salmonella serovars expressing the autofluorescent green-fluorescent protein were also located in the internal tissues of mung bean and alfalfa sprouts, respectively, after inoculation of seed or roots [38,45,46]. The mode of entry of bacterial human pathogens into plants remains unknown, but it is likely due to passive uptake at the site of injury where lateral roots emerge [46,48], as salmonella and *E. coli* O157:H7 have not been reported to excrete cell-wall-degrading enzymes (e.g., pectinases or cellulases) that might facilitate active entry. Pathogens may form biofilms on sprout surfaces and/or become part of biofilms produced by native microorganisms [49,50] (Figure 8.1).

FIGURE 8.1 Biofilm consisting of native bacteria on the surface of an alfalfa sprout hypocotyl.

8.3 INTERVENTIONS: SEEDS

8.3.1 Chemical and Physical

Sanitizing sprout seed presents a unique challenge in the arena of produce safety in that even a low residual pathogen population remaining on contaminated seed after treatment appears capable of growing to very high levels (up to 8 $\log_{10}$ CFU/g) due to favorable conditions of moisture, relative humidity, temperature, and nutrient availability during seed germination and subsequent sprout growth [51,52]. In addition, after a sanitizing procedure seed germination as well as sprout yield and quality need to be maintained at commercially acceptable levels. In 1999, based on research available at the time, the FDA published guidance documents recommending that commercial sprout growers treat sprout seed with one or more antimicrobial treatments such as 20,000 ppm of $Ca(OCl)_2$ that have been approved for reduction of pathogens on seeds or sprouts, with at least one approved antimicrobial treatment applied immediately before sprouting [53]. Also, in 2000 the FDA and the California Department of Health Services, Food and Drug Branch jointly released a food safety training video [54] for use by commercial sprout growers. The video, based on the FDA guidance documents, contains a recommendation to treat sprout seed with 20,000 ppm available chlorine from $Ca(OCl)_2$ for 15 minutes (continuous mixing) with potable water rinses both before and after seed treatment. Since sprout seed is considered a raw agricultural product, chemical seed treatments are subject to approval by the U.S. Environmental Protection Agency and not the FDA.

Population reductions reported after treatment of alfalfa seed artificially inoculated with salmonella or *E. coli* O157:H7 using 16,000 to 20,000 ppm of available chlorine has varied considerably among different laboratories, but usually are in the range of 2 to 4 $\log_{10}$ (Table 8.2). Lesser reductions were achieved after treatments with lower amounts of chlorine. A number of factors likely contribute to the variability in results. Such factors include the percentage of treated inoculated seed with broken, cracked, or wrinkled seed coats [81], differences in the initial pathogen population on the seed, the extent of mixing of sanitizer during treatment, the initial organic load on the seed, and the use of rinse steps before and after seed treatment. Some studies have been done with relatively low initial pathogen populations on the seed allowing for maximum population reductions of 2 to 3 $\log_{10}$. One consistent finding among the various laboratories is that the two pathogens when artificially inoculated onto sprout seed are not eliminated even by treatment with 16,000 to 20,000 ppm of available chlorine for 10 to 15 minutes.

The findings for similar studies with naturally contaminated seed are not consistent among laboratories (see below).

Investigations of recent foodborne outbreaks of salmonellosis due to contaminated sprouts indicates that treatment of sprout seed with high levels of chlorine by commercial growers reduces, but may not always eliminate, the risk of human illness [16–18,20]. The inability of seed treatments with high levels of chlorine to always ensure a pathogen-free seed under commercial practice may be due to several factors including the use of differing protocols for administering seed treatments at grower locations. Also, the particular seed treated, if naturally contaminated, may differ in the level of contamination present and the location of the pathogens on the contaminated seed (e.g., deep in cracks, crevices, and/or natural openings) (Figure 8.2). The ability of bacterial human pathogens to be internalized in seed under natural conditions in the field is not known, but seeds in general can harbor internalized native bacteria [82]. If present in internal tissues of the seed, pathogens may escape contact with chemical sanitizers.

Numerous chemical treatments in addition to chlorine as well as several physical treatments have been tested individually or in combination for eliminating pathogens from artificially inoculated sprout seed. To date there are few reports of stand alone chemical or physical interventions capable of eliminating pathogens from artificially inoculated sprout seed or consistently achieving the recommended 5 $\log_{10}$ reductions [4] without significant adverse affects on seed germination and/or sprout yield (Table 8.2). Most of the interventions included in Table 8.2 have been tested using more than the single set of conditions listed. Additional chemicals tested in the references cited, but not included in Table 8.2, are aqueous acetic acid, calcinated calcium, carvacrol, cinnamic aldehyde, citric acid, Citricidal® (NutriTeam, Inc., Reston, VT), CitroBio™ (= Pangermex) (CitroBio, Inc., Sarasota, FL), Environnè Fruit and Vegetable Wash™ (Consumer Health Research, Inc., Brandon, OR), ethanol, eugenol, linalool, methyl jasmonate, sodium carbonate, sodium hypochlorite, thymol, *trans*-anethole, trisodium phosphate, Tsunami 200® (Ecolab, Mendota Heights,

TABLE 8.2
Chemical and Physical Interventions for Reducing Pathogens on Inoculated Sprouting Seeds

Treatment	Conditions	Time	Seed type	Bacterium	Log reduction (CFU/g)	Seed germination	Ref.
Acetic acid, vapor	242 μl/l air, 45°C	12 h	Mung bean	Salmonella	>5, no survivors	No effect	55
Acetic acid, vapor	242 μl/l air, 45°C	12 h	Mung bean	*E. coli* O157:H7	>6, no survivors	No effect	55
Acetic acid, vapor	242 μl/l air, 45°C	12 h	Mung bean	*L. monocytogenes*	4.0	No effect	55
Acetic acid, vapor	300 mg/l air, 50°C	24 h	Alfalfa	Salmonella	0.8	No effect	56
Acidic EO water	1081 mV, 84 ppm chlorine	10 min	Alfalfa	Salmonella	1.5	No effect	57
Acidic EO water	1150 mV, 50 ppm chlorine	64 min	Alfalfa	*E. coli* O157:H7	1.6	Significant reduction	58
Acidic EO water	1079 mV, 70 ppm chlorine	15 min	Alfalfa	Salmonella	2.0	No effect	59
Allyl isothiocyanate	50 μl/950 cm^3 jar, 47°C	24 h	Alfalfa	*E. coli* O157:H7	>2.0, survivors present	Slight reduction	60
Ammonia, gas	300 mg/l	22 h	Alfalfa	Salmonella	2.0	No effect	61
Ammonia, gas	300 mg/l	22 h	Mung bean	Salmonella	5.0	No effect	61
Ammonia, gas	300 mg/l	22 h	Alfalfa	*E. coli* O157:H7	3.0	No effect	61
Ammonia, gas	300 mg/l	22 h	Mung bean	*E. coli* O157:H7	6.0	No effect	61
$Ca(OH)_2$	1%	10 min	Alfalfa	*E. coli* O157:H7	3.2	No effect	62
$Ca(OH)_2$	1%	10 min	Alfalfa	Salmonella	2.8–3.8	No effect	62, 63
$Ca(OCl)_2$	20,000 ppm	3 min	Alfalfa	*E. coli* O157:H7	>2.3, survivors present	Reduced rate	64
$Ca(OCl)_2$	20,000 ppm	10 min	Alfalfa	Salmonella	2.0	Slight reduction	63
$Ca(OCl)_2$	18,000 ppm	10 min	Alfalfa	Salmonella	3.9	No effect	65
$Ca(OCl)_2$	18,000 ppm	10 min	Alfalfa	*E. coli* O157:H7	4.5	No effect	65
$Ca(OCl)_2$	16,000 ppm	10 min	Mung bean	Salmonella	5.0	No effect	66
$Ca(OCl)_2$	16,000 ppm	10 min	Mung bean	*E. coli* O157:H7	3.9	No effect	66
Chlorine dioxide, acidified	500 ppm	10 min	Alfalfa	*E. coli* O157:H7	>2.4, survivors present	Significant reduction	64

(*continued*)

TABLE 8.2
Continued

Treatment	Conditions	Time	Seed type	Bacterium	Log reduction (CFU/g)	Seed germination	Ref.
Citrex™	20,000 ppm	10 min	Alfalfa	Salmonella	3.6	No effect	67
Citrex™	20,000 ppm	10 min	Alfalfa	*E. coli* O157:H7	3.4	No effect	67
Dry heat	50°C	60 min	Alfalfa	*E. coli* O157:H7	1.7	No effect	68
Dry heat	70°C	3 h	Alfalfa	Salmonella	3.0	Slight reduction	56
Fit™	According to label	15 min	Alfalfa	Salmonella	2.3	No effect	69
Fit™	According to label	15 min	Alfalfa	*E. coli* O157:H7	>5.4	No effect	69
H_2O_2	8%	3 min	Alfalfa	*E. coli* O157:H7	>2.9, survivors present	No effect	64
H_2O_2	8%	10 min	Alfalfa	Salmonella	3.2	No effect	63
Hydrostatic pressure	300 mPa	15 min	Garden cress	Salmonella	5.8	Reduced rate	70
Hydrostatic pressure	300 mPa	15 min	Garden cress	*Shigella flexneri*	4.5	Reduced rate	70
Lactic acid	5%, 42°C	10 min	Alfalfa	*E. coli* O157:H7	3.0	No effect	52
Radiation, gamma	Various		Alfalfa	Salmonella	*D*-value of 0.97 kGy	Dosage dependent	71
Radiation, gamma	Various		Alfalfa	*E. coli* O157:H7	*D*-value of 0.60 kGy	Dosage dependent	71
Radiation, gamma	Various		Broccoli	Salmonella	*D*-value of 1.10 kGy	Dosage dependent	72
Radiation, gamma	Various		Broccoli	*E. coli* O157:H7	*D*-value of 1.11 kGy	Dosage dependent	72
Sodium chlorite, acidified	1200 ppm, 55°C	3 min	Alfalfa	*E. coli* O157:H7	>1.9, survivors present	Slight reduction	64
Sulfuric acid	2 N	20 min	Alfalfa	*E. coli* O157:H7	5.0	No effect	73
Ozone, aqueous	21 ppm, w/sparging	64 min	Alfalfa	*E. coli* O157:H7	2.2	No effect	74
Ozone, aqueous	21.3 ppm, w/sparging	20 min	Alfalfa	*L. monocytogenes*	1.5	No effect	75
Pulsed UV light	5.6 J/cm^2, 270 pulses	90 sec	Alfalfa	*E. coli* O157:H7	4.9	Significant reduction	76
Dielectric heating, radio frequency	39 MHz, 1.6 kV/cm	26 sec	Alfalfa	Salmonella	1.7	No effect	77
Supercritical CO_2	4000 psi, 50°C	60 min	Alfalfa	*E. coli*, generic	1.0	No effect	78
Water, hot	3-stage: 25 to 50 to 85°C	30 min, 9 sec, 9 sec	Alfalfa	*E. coli*, generic	>4, no survivors	No effect	79
Water, hot	54°C	5 min	Alfalfa	Salmonella	2.5	No effect	34
Water, hot	80°C	2 min	Mung bean	Salmonella	>6	No effect	80

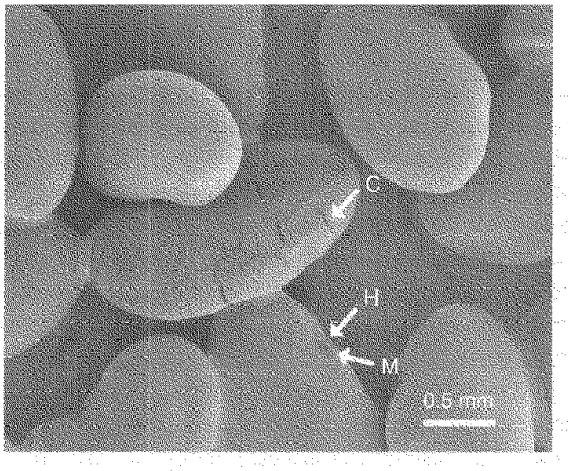

FIGURE 8.2 Scanning electron micrograph of alfalfa seeds showing extensive cracking of a seed coat and natural openings: C, crack in the seed coat; H, hilum; M, micropyle.

MN), Tween 80, Vegi-clean™ (Microcide, Inc., Detroit, MI), and Vortex® (Ecolab). Treating with aqueous chemicals at elevated temperatures can lead to greater reductions of pathogen populations on seed, but is often detrimental to seed germination [83]. Addition of high levels of the surfactant Tween 80 (1%, w/v) to 1% $Ca(OH)_2$ led to only an additional 1 $\log_{10}$ reduction or less in the population of salmonella on alfalfa seed [62,63]. Sonication of seed during treatment with aqueous antimicrobial compounds also did not have a significant effect, only slightly increasing the $\log_{10}$ kill obtained [68,83].

Treatment with gaseous acetic acid was reported to eliminate both salmonella and *E. coli* O157:H7, but not *Listeria monocytogenes*, from artificially inoculated mung bean seed without reducing seed germination [55]. Similar treatments of inoculated alfalfa seed led to either unacceptable reductions of seed germination [84] or were not effective [56]. Hot water treatments of alfalfa seed inoculated with generic *E. coli* were reported to eliminate the bacterium [79], but results both with alfalfa seed artificially inoculated with human pathogens as well as naturally contaminated seed have not been as promising due to lowered effectiveness and/or detrimental effects on seed germination [34,85]. Under commercial practice, the ability of hot water treatments to ensure consistent elimination of bacterial human pathogens from alfalfa seed was put into question by a recent multistate outbreak of salmonellosis due to contaminated alfalfa sprouts grown from seed treated with hot water followed by a soak in low levels (2000 ppm) of chlorine [20]. However, a recent laboratory study indicates that treatment of mung bean seed with hot water may be an effective seed-sanitizing step. Treatment of seed inoculated with salmonella at 55°C for 20 minutes, 60°C for 10 minutes, or 70°C for 5 minutes led to an approximate 5 $\log_{10}$ reduction [80]. Treating seed at 80°C for 2 minutes was even more effective resulting in an over 6 $\log_{10}$ reduction. None of

these temperature/time treatments led to a decrease in germination of the treated seed.

There have been a very limited number of studies on seed sanitization using naturally contaminated rather than artificially inoculated seed and these studies have evaluated the efficacy of hot water and chlorine treatments only [44,65, 85]. The use of naturally contaminated seed rather than artificially inoculated seed may give a more accurate prediction of the efficacy of seed treatments for eliminating bacterial human pathogens in commercial practice. This may be due to differences in bacterial populations per gram of seed (normally much lower on naturally contaminated seed than on artificially contaminated seed used for laboratory studies), possible differences in the location and physiological status of the pathogens and the potential presence of pathogens in biofilms. In contrast to studies with artificially inoculated seed treated with high levels of chlorine, research conducted independently in two laboratories using alfalfa seed lots naturally contaminated with salmonella indicated that treatment with chlorine (unbuffered and buffered to neutral pH, from 2,000 to 20,000 ppm) completely eliminated the pathogen [65,85]. However, a third laboratory published contrasting results using 20,000 ppm of unbuffered active chlorine also using seed naturally contaminated with salmonella [44]. The reasons for the differing results between laboratories may include differences in the degree of mixing during seed treatment as well as differences in the population and location of the pathogen on the particular naturally contaminated seed tested even if originating from the same seed lot.

Several physical treatments have also been tested for sanitizing sprout seed (Table 8.2). In 2000 the FDA approved exposure of sprout seed to ionizing radiation at doses up to 8 kGy [86]. Treatment with ionizing radiation can significantly reduce bacterial pathogens on sprout seed. Exposure of inoculated alfalfa seed to a 2 kGy dose of gamma irradiation led to a 3.3 and 2.0 $\log_{10}$ reduction in *E. coli* O157:H7 and salmonella populations, respectively, while still maintaining commercially acceptable yields as well as nutritive values of sprouts grown from the treated seed [71,87,88]. Higher dosages led to unacceptable reductions in yields. For alfalfa seed naturally contaminated with salmonella and treated with gamma radiation, Thayer *et al.* [89] reported a *D*-value of 0.81 kGy. An absorbed dose of 4 kGy was required to eliminate the pathogen, a dosage that results in significant reductions in yield. A required dosage of 4 kGy for pathogen elimination along with a *D*-value of 0.81 kGy indicates that individual naturally contaminated seeds may harbor pathogen populations in excess of 4 $\log_{10}$ CFU. Electron beam radiation or use of so-called soft electrons (low-energy electron beam, energies $\leq$300 kV) may also be useful for reducing pathogen populations on the surface of seed [90], but both have lowered penetration ability compared to gamma radiation.

Various treatment combinations (hurdle concept) for reducing contaminants on sprout seed have also been tested. Bari *et al.* [68] reported that the combination of dry heat (50°C, 1 hour) followed by treatment with hot acidic electrolyzed oxidizing (EO) water and sonication was able to reduce populations of *E. coli* O157:H7 on artificially inoculated mung bean seed by 4.6 $\log_{10}$

but the combination treatment was less effective when tested against inoculated radish and alfalfa seed. Seed germination and subsequent sprout growth were not adversely affected. In the same study, a dry heat (50°C, 1 hour) seed treatment in combination with exposure to 2 to 2.5 kGy of gamma radiation led to the elimination of the pathogen on mung bean, radish, and alfalfa seed, but resulted in decreases in yield, most significantly for mung bean and radish. Lang *et al.* [52] found that successive treatments of alfalfa seed artificially inoculated with *E. coli* O157:H7 with lactic acid and chlorine (2000 ppm) were slightly more effective than lactic acid treatments alone, but were less effective than high levels of chlorine (20,000 ppm). Sharma *et al.* [91] found that treating alfalfa seed inoculated with *E. coli* O157:H7 first with ozone (continuous sparging in water) followed by a dry heat treatment (60°C, 3 hours) led to a greater than 4 $\log_{10}$ reduction of the pathogen population, but survivors were detected by enrichment. A sequential washing treatment with thyme oil (5 ml/l) followed by ozonated water (14.3 mg/l) and aqueous ClO_2 (25 mg/l) led to a 3.3 $\log_{10}$ reduction of *E. coli* O157:H7 on inoculated alfalfa seed [92].

The large body of research reported subsequent to the release of the FDA guidance documents [53] indicates that several alternative chemical and physical treatments may be similar or greater in efficacy to high levels of chlorine for reducing pathogen populations on sprout seed. For sanitizing alfalfa seed such treatments include seed soaks in 1% $Ca(OH)_2$, 1% calcinated calcium, FIT®, 8% H_2O_2, or 2% CITREX™ [62,63,67,69,83,93]. For sanitizing mung bean seed exposure to gaseous acetic acid or soaking seed in hot water appear especially promising [55,80]. The efficacy of these alternative chemical and physical treatments needs to be confirmed by other researchers ideally using naturally contaminated seed. In contrast to high levels of chlorine, several of these alternative methods of sanitizing seed may be acceptable for use by organic growers as well as conventional growers pending any required regulatory approvals. The cost of some of these alternative methods to the commercial grower may be prohibitive, however. Cost may not be as much of an issue for home growers.

8.3.2 Biological

In contrast to the voluminous literature concerning biological control of plant pathogens [94] as well as numerous studies on the biological control (competitive exclusion) of pathogens in poultry, meat, and dairy products [95,96], there is little published information on the use of antagonistic microorganisms to control human pathogens on produce. The ideal biocontrol product for use on sprout seed and sprouts would contain a nonpathogenic microorganism(s) that is genetically stable, easily cultured and formulated using low-cost substrates and materials, has a long shelf life, is easily applied to seeds and/or sprouts, is highly effective on a variety of sprout types and against several human pathogens, and is affordable for the grower. For control of pathogens in poultry, meat, and dairy products, single microbial strains or defined or undefined consortia of microbes have been tested as antagonists.

Most of the studies on biological control of bacterial human pathogens on produce have examined the use of lactic acid bacteria (LAB) as antagonists [95]. LAB are attractive candidates for commercial biological control agents due to their common occurrence on sprout surfaces [41,97], their ability to produce multiple antimicrobial agents including bacteriocins, hydrogen peroxide, and organic acids *in vitro*, their extensive use in the food industry for fermentation, and their lack of known pathogenicity [95]. A strain of *Lactococcus lactis* inhibitory *in vitro* against *Listeria monocytogenes* due to acid production was tested for control of the pathogen when the two bacteria were co-inoculated onto alfalfa seed before sprouting [98]. Results indicated that the strain was much less inhibitory towards the pathogen *in situ* than *in vitro*, reducing pathogen populations on the sprouts by only 1 $\log_{10}$. In a second study on LAB, Wilderdyke *et al.* [99] found that of 58 isolates of LAB isolated from alfalfa seeds and sprouts, 32 were inhibitory towards the three pathogens salmonella, *E. coli* O157:H7, and *Listeria monocytogenes* in agar spot tests. One strain of *Lactococcus lactis* subsp. *lactis* was particularly inhibitory towards all three pathogens on agar media and in broth culture. The same group reported a significant reduction in populations of *Listeria monocytogenes* on alfalfa sprouts after application of a strain of LAB in the seed soak solution [100]. A commercial product containing a lactic acid bacterium is available in Japan for controlling *E. coli* O157:H7 on Daikon radish sprouts [101]. This product is to be sprayed onto seeds and sprouts several times during the sprouting process. In our laboratory, we have tested hundreds of plant-associated bacteria, primarily isolated from sprout surfaces, for their ability to inhibit growth of salmonella inoculated to alfalfa seed in small-scale laboratory bioassays [102]. Of these, a few isolates (none are LAB) have been identified that consistently reduce growth by several $\log_{10}$ units in small-scale laboratory bioassays. Currently, the effectiveness of these antagonists is being evaluated in larger scale experiments and studies on their mode of action are also underway.

More than a single antagonist may be required for controlling pathogens on germinating seeds of various sprout types due to compositional differences in the native microflora [103]. Treating artificially contaminated alfalfa seed with a novel purified bacteriocin, colicin HU194, led to reductions ranging from 3 $\log_{10}$ CFU/g to complete elimination of *E. coli* O157:H7. Efficacy was dependent on the particular strain of *E. coli* O157:H7 used for seed inoculation [104]. Bacteriophages are also being researched as a possible antimicrobial intervention for application to sprout seed [105]. Biological control agents may also be useful for reducing spoilage caused by soft-rotting bacteria [106].

8.4 INTERVENTIONS: SPROUTS

A variety of antimicrobial chemicals have been tested as additives to sprout irrigation water for the purpose of preventing or reducing the growth of native microflora and bacterial human pathogens. A study in our laboratory indicated that addition of H_2O_2, Tsunami®, acidified $NaClO_2$, Aquatize™ (Bioxy, Raleigh, NC), EDTA, $NaPO_4$, and NaOCl at varying concentrations

to the irrigation water did not reduce the populations of the native microflora on alfalfa sprouts grown in a commercial-scale tray system by more than approximately 1 $\log_{10}$ without evidence of phytotoxicity [107]. Piernas and Guiraud [108] reported that spray irrigation of tray-grown rice sprouts with chlorinated water (100 mg/l) every 6 hours was not effective in reducing populations of total aerobic bacteria, *B. cereus*, or *L. innocua*. Daily spraying of alfalfa sprouts grown from artificially inoculated seed with chlorine (100 mg/l) led to reduction of less than 2 $\log_{10}$ in the population of salmonella at day 4 of sprouting [51]. Daily irrigation with ClO_2 (100 mg/l) did not reduce the population of total aerobic bacteria on alfalfa sprouts grown in trays, but did reduce populations of *V. cholera* up to 2 $\log_{10}$ when sprouts were grown from seed inoculated with the pathogen [32]. A reduction of 4 $\log_{10}$ for total coliforms was obtained for mung bean sprouts that were subject to irrigation with 0.2 ppm gaseous ozone and 0.3 to 0.5 mg/l of ozonated water at days 4 to 7 of sprouting [105]. Rinsing of inoculated alfalfa seed growing in plastic jars with aqueous ClO_2 (25 mg/l) or ozonated water (9.27 mg/l) after 48 or 72 hours of sprouting was ineffective in reducing populations of *E. coli* O157:H7 [92]. However, rinsing with thyme oil (5.0 mg/l) alone or in sequence with ClO_2 and ozonated water led to reductions of up to 2 $\log_{10}$ in pathogen populations when carried out at 24 and 48 hours into the sprouting process. None of the rinsing treatments were effective at 72 hours, however. Rinsing with water was ineffective at all time points. Taormina and Beuchat [110] tested a variety of aqueous antimicrobial chemicals as spray treatments for reducing or eliminating *E. coli* O157:H7 from the surface of growing alfalfa sprouts. None of the chemicals were effective for reducing pathogen populations and only acidified $NaOCl_2$ (1200 ppm) controlled the growth of the pathogen. A complication of addition of antibacterial compounds to the irrigation water is that any pathogens present in the spent irrigation water may be killed, but viable pathogen populations may remain on the sprouts rendering the testing of spent irrigation water for viable pathogens meaningless [4].

Several postharvest treatments for reducing the populations of native microbes and pathogens have been examined. Water rinses are not highly effective in reducing microbes on sprouts with resultant population reductions of 1 $\log_{10}$ or less [31,39,110,111]. A 2-minute treatment with aqueous ozone (23 ppm) did not reduce the population of aerobic microorganisms on alfalfa sprouts [75]. Dipping in hot water (60°C) for 30 seconds led to a reduction of 2 $\log_{10}$ in the population of total microbes on soybean sprouts [112] and a similar treatment for 5 minutes led to a reduction of 5 $\log_{10}$ in aerobic plate counts on rice sprouts [113]. Blanching in hot water (90°C, 1 minute) was reported to reduce microbial counts by 5 $\log_{10}$ units for mung bean sprouts [111]. Rinsing of mung bean sprouts in 1 and 2% lactic or acetic acid reduced the native microflora by less than 2 $\log_{10}$ [111]. Treatment of rice sprouts with chlorine (100 mg/l) for up to 10 minutes decreased aerobic plate counts by only 1.5 $\log_{10}$ [111]. Treatment (10 minutes) of inoculated mung bean sprouts with chlorous acid ($HClO_2$; 268 ppm), NaOCl (200 ppm), or lactic acid (2%) resulted in a maximum reduction of 1 $\log_{10}$ for total aerobes [114].

Blanching in hot water (100°C, 30 seconds) did not eliminate *E. coli* O157:H7 from alfalfa sprouts [115]. Treatment (10 minutes) of alfalfa sprouts with EO water (84 mg/l of available chlorine) in conjunction with sonication led to a reduction of 1.5 $\log_{10}$ in the population of salmonella [116]. Treatment (64 minutes) with EO water (50 mg/l of available chlorine) resulted in a reduction of 3 $\log_{10}$ of *E. coli* O157:H7 on alfalfa sprouts without any reported changes in appearance [117]. Aqueous ozone treatments (maximum concentration of 20 to 23 ppm, treatment time of 20 to 64 minutes) of alfalfa sprouts led to a maximum population reduction of approximately 1 to 2 $\log_{10}$ for *L. monocytogenes* and *E. coli* O157:H7, respectively [75,118]. The greatest log reductions reported for a postharvest aqueous chemical treatment were for $HClO_2$. Treatment (10 minutes) of inoculated mung bean sprouts with $HClO_2$ (268 ppm) resulted in a reduction of approximately 5 $\log_{10}$ of salmonella and *L. monocytogenes*. Lactic acid (2%) was also tested in this study, but was less effective [114]. Exposure of inoculated alfalfa sprouts to gaseous acetic acid or allyl isothiocyanate vapor led to significant reductions in the population of salmonella, but also led to undesirable changes in sensory quality [56].

Most likely the only postharvest treatment able to inactivate pathogens that have been internalized into sprouts during the growing process is irradiation. A postharvest treatment with gamma radiation at 2 kGy extended the shelf life of alfalfa and broccoli sprouts by 10 days due to significant decreases in the native microflora [72,88]. Doses up to 2.6 kGy did not significantly change the appearance or nutrient quality of alfalfa sprouts [118]. Salmonella was eliminated from alfalfa sprouts grown from naturally contaminated seed when exposed to gamma radiation at a minimum dose of 0.5 kGy [120]. Irradiation of inoculated alfalfa sprouts with 3.3 kGy of beta radiation (electron beam) eliminated *L. monocytogenes* without an adverse effect on quality [121].

8.5 REDUCING THE RISK OF FUTURE OUTBREAKS

Several steps can be taken to minimize the risk of future sprout-related outbreaks of foodborne illness including the use of good agricultural practices (GAPs) during the production of sprouting seed as detailed in several recent government, university, and produce organization publications [122–124]. Sprout seed is obtained from plants grown in the open field and thus subject to potential contamination by nonpotable irrigation water, manure, domestic and wild animals, birds, farm machinery, and farm workers. To the author's knowledge, there are no fields in the U.S. or elsewhere designated solely for the production of seed destined for use by sprout growers. Settings on harvesting machinery should be such as to minimize damage to the seed. Cross contamination between clean and contaminated lots of harvested seed can occur in seed cleaning (conditioning) facilities and also when lots of seed are mixed before packaging and distribution. Several salmonella serovars were detected in the waste streams of a seed-cleaning machine in a U.S. alfalfa seed-cleaning facility indicating the presence of salmonella in the local alfalfa fields where

the seed originated [125]. Seed-cleaning machines should be thoroughly cleaned and sanitized before and between lots of seed destined for sprouting. Seed scarification has been used historically to increase the germinability of seed lots that contain a significant amount of hard seed. Scarification involves the mechanical abrasion of the seed coat to allow for entry of water facilitating germination. Damage to the seed coat may make elimination of bacterial pathogens by treatment with chemical sanitizers more difficult [62,81] and probably should be avoided if possible. There is also the potential for contamination during transit and storage of seed as well as during seed germination, growth, and harvest.

Commercial sprout growers need to follow good manufacturing practices (GMPs) and have written standard sanitation operating procedures (SSOPs) and a hazard analysis and critical control point (HACCP) plan in place [126]. Growers should be thoroughly familiar with the recommendations contained in the FDA guidance documents which include detailed methods for testing of spent irrigation water for salmonella and *E. coli* O157:H7 [53]. Seed should be of high quality and all bags of seed should be inspected for evidence of rodent activity (gnawed holes and presence of urine stains using a blacklight). Thorough testing of all lots of sprout seed for bacterial pathogens is desirable and should reduce the risk of sprout-related outbreaks of foodborne disease. A sampling and testing protocol for use with sprout seed for human pathogens has been proposed [127]. However, due to the sporadic and low level of contamination with human pathogens often encountered, a negative sample test cannot guarantee that the entire lot is pathogen free. Thus, an effective, approved seed-sanitizing step should be applied by the grower, and the spent irrigation water or sprouts should be tested for the presence of pathogens. Irrigation water needs to be of high quality and the use of well water also requires regular testing for adequate levels of residual chlorine. Postharvest contamination of sprouts can occur during transit, storage, display, and by cross contamination in restaurant or home kitchens and adequate precautions need to be taken.

8.6 RESEARCH NEEDS

Despite considerable research efforts towards the development of sprout seed-sanitizing methods there is still a need for highly effective, low-cost, easily implemented, and environmentally benign seed-sanitizing strategies that can be used by organic and conventional sprout growers. The use of 20,000 ppm $Ca(OCl)_2$ presents worker and environmental safety concerns, may not always be effective in eliminating human pathogens from contaminated seed lots under commercial practice, and can be highly detrimental to the germination capacity of some seed types [65]. The potential for internalization of bacterial human pathogens into sprouts during germination and growth from contaminated sprouting seed has been demonstrated, but the location of pathogens on naturally contaminated seed is still not known. Are the pathogens solely surfaceborne, sometimes entering into cracks and natural openings such as

the hilum and micropyle in the seed coat, or are they also present internally in the seed coat as are some seedborne plant pathogenic bacteria [128]? The optimization and commercialization of biological control agents for use on sprouting seed as an alternative to chemical sanitizers is highly desirable.

The ecology of human pathogens on sprouts is not well defined and several questions remain unanswered. Are pathogens capable of forming biofilms on sprout surfaces or can they become part of biofilms formed by the native microflora making their eradication more problematic? What microbial cell surface components (e.g., curli, fimbriae, flagella, and extracellular polysaccharides such as colanic acid and cellulose) are important for the initial attachment to plant surfaces and subsequent biofilm formation? Does the plant react in any way to the presence of pathogens on surfaces or in internal tissues? Could sprout seed cultivars be developed that release high levels of antibacterial compounds upon germination that might inhibit growth and survival of pathogens?

Further research in the areas mentioned above should assist in the development of improved strategies for reducing the risk of future foodborne outbreaks allowing for greater consumer confidence in the microbiological safety of sprouts and ensuring the survival of a strong sprout industry worldwide. Intervention strategies developed for seeds and sprouts may also be applicable to ensuring the microbiological safety of other types of produce.

ACKNOWLEDGMENTS

Sincere thanks are due to Drs. Francisco Diez-Gonzalez and Mindy Brashears for supplying articles in press, to Drs. Jeff Farrar and Glenn Henderson for supplying information concerning sprout-related outbreaks, and to Drs. Jeff Farrar, James Smith, and Mary Lou Tortorello for critically reviewing the manuscript before publication.

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